

bioenergetics and thermogenesis, the effects of insulin administration on complex I and UCP1 expression and cristae remodeling in BAT mitochondria were investigated in this study. Two months old Wistar rats were fed with standard pelleted food ad libitum. The rats were divided into three groups, each with six animals. The first two groups were treated with low (0.4 IU/kg) or high (4 IU/kg) dose of insulin i.p. (Novo Nordisk, Denmark), while the group treated with 0.9% saline solution served as control. The interscapular portion of BAT was used for Western blot and ultrastructural analysis. Western blot analyses were performed using primary antibodies against NDUFA9 and UCP1 (Abcam, UK). Samples for electron microscopy were routinely embedded in Araldite. Ultrathin sections were contrasted and examined on a Philips CM12 transmission electron microscope. Obtained electron micrographs were used for ultrastructural analysis of mitochondria and stereology (cristae volume density). Western blot analyses showed decrease of relative protein expression of NDUFA9 and increase of relative protein expression of UCP1 in treated groups. At ultrastructural level, changes in mitochondrial morphology can be observed. Namely, insulin induced extensive cristae remodeling in both treated groups, increasing cristae abundance e.g. cristae volume density. These results indicate that insulin can modify cristae structure in BAT mitochondria by decreasing complex I expression and increasing UCP1 expression and their incorporation. \*This work is supported by grants #173054 and #173055 of MPNTR (Serbia); and by "Hubert Curien/Pavle Savic" Partnership — bilateral cooperation between Serbia and France.

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## S2.P8

### Effects of cell division activity, mitochondrial energy status and inhibition of mitochondrial fission on cell viability and distribution of native and mutant version of huntingtin in rat tissue culture model of Huntington's disease (PC12 cells)

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Huntington disease (HD) is an autosomal-dominant neurodegenerative disorder characterized by a selective loss of neurons, especially from the striatum and deep layers of cerebral cortex. It belongs to polyglutamine expansion diseases because it is caused by an increase in the number of glutamine codon (CAG) in exon 1 of the gene encoding the protein huntingtin (Htt). The number of the codon higher than 35 results in a mutated version of the protein (mHtt) that contains an abnormal stretch of over 35 glutamines at the N terminus. The clinical symptoms usually occur between 30 and 40 years of age. No therapeutic strategies capable of halting or delaying the disease progression have yet been proposed. Mitochondria play a vital role in HD pathogenesis. Available data indicate that mitochondrial defects initiate disease onset. Accordingly, two categories of phenomena are regarded to precede other HD symptoms, namely changes of mitochondrial energy status and changes of their fusion and fission. Therefore analysis of relationships between mitochondrial energy status and mitochondrial dynamics appears to be a logical step in elucidation of mitochondrial

dysfunction in HD pathomechanism. We performed experiments on PC12 cells (derived from a pheochromocytoma of the rat adrenal medulla) with expression of exon 1 of huntingtin encoding gene containing 23 (Htt) or 74 (mHtt) repeats of glutamine codon, namely PC-12HD-Q23 and PC-12HD-Q74, respectively. Htt and mHtt expression was induced by doxycycline and monitored due to GFP labeling. PC12 cycling cells were differentiated into post-mitotic neuron-like cells upon treatment with the nerve growth factor (NGF). This enabled studies on cells differing in division activity and level of differentiation. We also applied media with different concentrations of glucose to affect mitochondrial energy status within intact cells as well as an inhibitor of Drp1, i.e. Mdivi-1 to estimate the impact of mitochondrial fission. The analysis of Htt and mHtt distribution and cell viability under the applied conditions was performed by confocal microscopy. Mitochondria were labeled due to transformation by pDsRed2-Mito Vector. The obtained results indicate that the presence of NGF and Mdivi-1 distinctly influences the levels of Htt and mHtt expression and distribution as well as the viability of PC12 cells. Therefore they contribute to the understanding of mitochondria role in HD etiology. The studies were supported by the grant: NCN 2011/01/B/NZ3/00359.

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## S2.P9

### Molecular motor protein kinesin-1 controls the localization of mitochondria and myofibrils components

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Proper distribution of mitochondria in cells is important for cell functions. Here, we show that a molecular motor, kinesin-1 heavy chain Kif5b plays important roles in anterograde transport of mitochondria, alpha-sarcomeric actin, non-muscle myosin IIB, together with intermediate filament proteins' desmin and nestin in the elongating myotubes. Mice with Kif5b conditionally knocked out in myogenic cells showed aggregation of mitochondria, actin filaments and intermediate filament proteins in the differentiating skeletal muscle cells, which further affected myofibril assembly and their linkage to the myotendinous junctions. The expression of Kif5b in mutant myotubes rescued the localization of mitochondria and the affected proteins.

## Reference

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